



**Karolinska
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Institutionen för Cell- och Molekylärbiologi

Studies of cohesin functions in the yeast and human DNA damage response

AKADEMISK AVHANDLING

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Elin Enervald

M.Sc.

Huvudhandledare:

Doktor Lena Ström
Karolinska Institutet
Institutionen för Cell- och Molekylärbiologi

Bihandledare:

Professor Christer Höög
Karolinska Institutet
Institutionen för Cell- och Molekylärbiologi

Fakultetsopponent:

Doktor Christian Haering
European Molecular Biology Laboratory (EMBL)
Structural and Computational Biology Unit, Heidelberg

Betygsnämnd:

Professor Stefan Åström
Stockholms Universitet
Wenner-Gren Institutet
Utvecklingsbiologi

Docent Olle Sangfelt
Karolinska Institutet
Institutionen för Cell- och Molekylärbiologi

Docent Marianne Farnebo
Karolinska Institutet
Institutionen för Onkologi/Patologi

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ABSTRACT

Maintaining genome stability is critical to cell survival and normal cell growth and most human cancers display some form of genome instability. Genome instability is caused by multiple reasons and the ability to properly recognize, signal and subsequently repair DNA damages is crucial. DNA double-strand breaks (DSBs) are considered as the most toxic type of DNA lesion and therefore the ability to accurately repair these breaks are of outmost importance.

The cohesin complex is a large DNA-binding complex with numerous functions vital for maintaining genome integrity. The canonical role of the cohesin complex is to mediate cohesion between the sister chromatids from the time they are generated to their separation in mitosis. However, cohesin has over the years been assigned with additional functions independent on cohesion, such as regulation of gene transcription, DSB repair and activation of DNA damage checkpoints.

We have investigated the role of cohesin and its loading partner NIPBL, in the cellular responses to DSBs, using human cell cultures and budding yeast as model systems.

There are two main mechanisms used to repair DSBs, homologous recombination (HR) and nonhomologous end joining (NHEJ). More recently, a new “alternative” pathway for DSB repair has emerged, termed alternative end joining (A-EJ). By studying B-cells derived from patients with Cornelia de Lange Syndrome, we have observed a strong correlation between heterozygous loss-of-function mutations in the *NIPBL* gene and a shift towards the use of the microhomology-based A-EJ mechanism for DSB repair during class switch recombination. Furthermore, the early recruitment of 53BP1 to DSBs was reduced in the NIPBL-deficient patient cells. Our results suggest that NIPBL plays an important role for NHEJ, potentially by regulating DNA end resection.

In budding yeast postreplicative cells, the cohesion is reactivated in response to a DSB. This reactivation includes additional Scc2-dependent loading of cohesin to the region around the DSB and formation of new cohesion, both proximal to the DSB and on undamaged areas of the genome. This phenomenon is known as damage-induced (DI) cohesion. By analyzing the role of DNA polymerase η in DI cohesion we discovered that establishment of DI cohesion at the vicinity of a DSB and on undamaged chromosomes, genome wide, are regulated differently. We concluded this based on our finding that Pol η is required for genome-wide DI cohesion while it is dispensable for S phase cohesion and DSB-proximal cohesion. Cohesion establishment, both during S phase and following a DSB, depend on the acetylation activity of the highly conserved acetyltransferase Eco1. Using *in vitro* studies, we found that Pol η is an Eco1 substrate. In addition, we provide results suggesting that Eco1 acetylation of Pol η regulates its activity in DI-cohesion.

All together, these studies highlight the importance of cohesin, and its regulators, for genome stability. Future investigations, aimed at addressing the different mechanisms by which cohesin functions in the DNA damage response will most likely advance our understanding of how genome stability is maintained.